RESEARCH ARTICLE

Therapeutic Effectiveness of Zinc and Copper Alone and in Combination with Enrofloxacin for the Treatment of Sub-Clinical Mastitis in Dairy Buffaloes

Muhammad Imran Latif1, Tanveer Ahmad1, Ghulam Muhammad1, Muhammad Kashif Saleemi2*, Muhammad Junaid Iqbal1, Arfan Yousaf3, Masood Akhtar4 and Zeeshan Akbar

1Department of Clinical Medicine and Surgery
2Department of Pathology University of Agriculture, Faisalabad, Pakistan
3Department of Veterinary Basic Sciences, PMAS-Arid Agriculture University Rawalpindi, Pakistan; 4Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan

ARTICLE INFO
Received: Mar 09, 2014
Accepted: Apr 28, 2014
Online: Apr 30, 2014

Keywords
Buffalo
Mastitis
Copper
Zinc
Enrofloxacin

ABSTRACT
The present study was designed to compare the therapeutic effectiveness of zinc (Zn) and copper (Cu) alone and in combination with enrofloxacin for the treatment of sub-clinical mastitis in dairy buffaloes. Out of 152 lactating buffaloes, 45 suffering from sub-clinical mastitis were selected on the basis of positive Surf Field Mastitis Test (SFMT). These buffaloes were randomly divided into five equal groups (A-E). Buffaloes of group A were supplemented with ZnSO4 @ 1 g per animal per day, Group B CuSO4 @ 1 g per animal per day for 14 days. Group C was injected with enrofloxacin @ 2.5 mg/kg B.W through intramuscular route for 5 consecutive days. Buffaloes of group D were administered with intramuscular injection of enrofloxacin @ 2.5 mg/kg B.W for five consecutive days along with the supplementation of ZnSO4 (1 g per day) and CuSO4 (1 g per day) for 14 days. Group E was kept as untreated control. Milk and blood samples were collected at day 0, 7 and 14 post treatment for various tests like somatic cell count (SCC), SFMT based and bacteriological based cure rates, total leukocyte count (TLC), lymphocyte percentage, serum zinc and copper concentration. The SCC in milk decreased significantly (P<0.01) in combined therapy group D at day 14 post treatment. Serum zinc and copper concentrations increased significantly (P<0.01) in supplemented groups at day 7 and 14 post treatment. TLC and lymphocytes percentage was significantly higher (P<0.01) in combined therapy at day 7 and 14 post treatment. Percent cure rate on the basis of SFMT and bacteriological based cure rate was higher in group D. Milk yield of affected quarter was improved significantly (P<0.01) in the group D and other groups showed non-significant (P>0.05) improvement at day 14 post treatment. It may be concluded that the combined therapy was better than alone therapy of zinc sulphate, copper sulphate and enrofloxacin.

INTRODUCTION
Pakistan is gifted with huge livestock population. The livestock sector shares 55.1% of the agricultural GDP. There are nearly 31.7 million buffaloes in Pakistan which produces 28.694 million tons of milk annually. Buffaloes share 61.8% of the total milk production in our country (Anonymous, 2013). Buffalo is called ‘the black gold’ of Pakistan (Bilal et al., 2006). Among the dairy diseases, mastitis is one of the most important dairy diseases in Pakistan (Akram, 2002). Mastitis occurs in two forms viz., clinical and sub-clinical (Radostits et al., 2007). Mastitis leads to huge economic losses in Pakistan (Ahmad, 2001). Economical loses are in the form of decrease in milk production and increase in cost of medication (Degraves and Fetrow, 1993). In sub-clinical mastitis, the apparent signs are absent but somatic cell count (SCC) is high (Radostits et al., 2007). Sub-clinical mastitis results in culling of large number of animals, increased treatment cost, decreased
milk production and poor quality milk (Dobbins, 1997). Sub-clinical mastitis causes more economical losses because it is 3 to 4 times more prevalent than the clinical mastitis (Jasper et al., 1982). Mastitis leads to reduction in the number of B- lymphocytes in the blood which indicates immunosuppression (Ishikawa and Shimizu, 1982). The subclinical mastitis also resulted in increased level of serum haptoglobin and serum amyloid A (Nazifi et al., 2011).

Zinc plays a vital role in different functions of immune system. It directly induces monocytes to produce interleukin 2, 6 and tumor necrosis factor alpha (TNF α) which act as the pro-inflammatory mediators (Rink and Kirchner, 1998). Zinc is a cofactor for many proteins and enzymes which take part in the initial response of body to any infection or inflammation (Prasad, 1979). Superoxide dismutase which requires Zn for activation is an enzyme which protects the cellular membranes of udder and teat against the activity of superoxide radicals (Moynahan, 1981). Deficiency of Zn causes decrease in basal metabolic rate of ruminants and also leads to weaken skin and epithelium (Harmon and Torre, 1994). The cows which receive Zn supplementation are comparatively healthier than those animals which are not receiving Zn supplementation (Chirase et al., 1991).

Copper is also an important element for the proper functioning of immune system. (Failla, 2003). This element plays a vital role in the formation of neutrophils and also influences the phagocytic activity of macrophages (Linder, 1991). Deficiency of Cu causes decrease in number of circulating neutrophils, B cells and T cells. Animals which are deficient in Cu are highly prone to infections (McDowell, 2005).

When Cu is given along with Zn, it plays an important role in the activity of superoxide dismutase and also helps in excretion of free radicals. Ceruloplasmin has an anti-inflammatory activity, thus may be helpful to control mastitis (Tomlinson et al., 2008). Excessive supplementation of Zn results in secondary Cu deficiency. So, dietary Zn must not be more than five times the dietary Cu (National Research Council, 2001). Pakistani soil is deficient in available elements like Zn, phosphorus and nitrogen (Alam et al., 1997). Majority of the forages available at livestock station Khizerabad, Sargodha, Pakistan are deficient in Zn, Cu and cobalt (Khan et al., 2009). Deficiency of Zn, Cu, iron and selenium has been reported in Nili-Ravi buffaloes at Buffalo Research Institute, Pattoki, District Kasur, Pakistan (Akhtar et al., 2009).

Enrofloxacin (Encure-10™, Nawan Laboratories Pvt. Limited, Karachi, Pakistan) showed remarkable decrease in SCC and total bacterial count in bovine sub-clinical mastitis treatment (Reena and Dash, 2003). Enrofloxacin had 83.3% recovery rate in bovine sub-clinical mastitis. As there is immunosuppressive effect of mastitis and immunomodulatory effect of Zn and Cu, therefore, present study was planned with objective to Compare the therapeutic efficacy of Zn and Cu alone and in combination with enrofloxacin in the treatment of sub clinical mastitis.

MATERIALS AND METHODS

Out of 152 lactating buffaloes, 45 suffering from sub-clinical mastitis were randomly selected on the basis of positive SFMT (Muhammad et al., 2010) from three dairy herds having almost similar management conditions with slight variation in feeding regime in Faisalabad. Animals of each dairy herd were further divided into five equal groups (A, B, C, D and E). Guidelines of International Dairy Federation (Thorburn, 1990) for mastitis therapy were followed for assigning study animals in different groups.

Milk samples were collected aseptically from all the experimental animals 3 times i.e. at day 0, day 7 and 14 of treatment plan. The milk samples were cultured on blood agar for bacteriological examination and SFMT performed and the reactions generated were interpreted as described by Schalm et al. (1971). A quarter was considered to be infected if 5 or more similar colonies were present on the plate. The representative colonies were isolated and purified by streaking on fresh blood agar plates. After isolation, Gram staining was performed. Confirmation was done by catalase test and coagulase test. Catalase positive, coagulase positive and gram positive coecal isolates were presumptively identified as Staphylococci or Micrococci. Organisms other than Staphylococcus were identified by routine biochemical tests (National Mastitis Council Inc., 1990).

Gram staining was performed as (Cruckshank et al., 1975) and slides were examined under oil immersion lens of microscope. Gram positive cocci were tested for catalase production and coagulase test was performed to divide Staphylococcal isolates into coagulase positive Staphylococci (CPS) and coagulase negative Staphylococci (CNS). Procedure described by National Mastitis Council Inc. (1990) was followed. From milk samples of all the experimental animals SCC determined before treatment and on day 14 post treatment. Newman’s Lampert staining was used for SCC in milk and Stained slides were examined under an oil immersion lens. It was carried out by adopting the technique described by Schalm et al. (1971). Copper and zinc concentration in the serum of all the experimental animals were determined before treatment, on day 7 and 14 post treatment. Wet digestion of serum samples was carried out as described by Richard (1969).
Total leukocyte count (TLC) and lymphocyte percentage was carried out at day 0 (control) and day 14 post treatment by Medonic M series (MERCK, Stockholm, Sweden). Daily milk yield of every animal was calculated from day 0 (control) today 14 post treatment. Effect of treatments on milk production was compared.

Statistical analysis
The data thus obtained was analyzed by randomized complete block design (RCBD) and means were compared by Duncan’s multiple range test (DMR) and Least significant difference test (LSD) using SAS statistical software (SAS, 2004).

RESULTS

Effect of treatments on somatic cell count in subclinically mastitic buffaloes
Mean somatic cell count of zinc sulphate, copper sulphate and enrofloxacin combined therapy (group D) was significantly lesser than that of group A, B and C. Cumulative mean of zinc sulphate was nonsignificant (P>0.05) with the enrofloxacin treated. Decrease in somatic cell count is an indicator of good health of the mammary glands. Maximum decrease in SCC was seen in the group which was treated with combined therapy of zinc sulphate, copper sulphate and enrofloxacin (group D) Table 1.

Effect of treatments on bacteriological cure rate in subclinically mastitic buffaloes
Cure rates based on bacteriological examination at day 14 post treatment is given in Table 1. The combined therapy of all three zinc, copper and enrofloxacin (group D) had highest bacteriological cure rate of 80%, followed by zinc sulphate (group A) with 65%, enrofloxacin (group C) 53% and copper sulphate (group B) 40%. So the combined therapy of all three (group D) was superior to the zinc sulphate alone and copper sulphate alone therapy Table 2.

Effect of treatments on Surf Field Mastitis Test based cure rate
Mean Surf Field Mastitis Test based cure rate has given in Table 2. Mean SFMT score of combined treatment (group D) was significantly lower than the mean SFMT score of zinc sulphate (group A), copper sulphate (group B) and enrofloxacin (group C). Among the different treated groups, the group D showed the highest cure rates percentage on both days of performing SFMT Table 3.

Effect of treatments on milk production of affected quarter
Effect of the treatment on milk yield of the affected quarter was recorded from day 0 to day 14 post treatment. Milk yield of zinc sulphate, copper sulphate and enrofloxacin combined therapy (group D) was significantly higher (P<0.01) than the zinc sulphate alone (group A), copper sulphate alone (group B) and enrofloxacin alone (group C). At day 14 post treatment, total milk production of copper sulphate treatment (group B) was non-significant (P>0.05) with the enrofloxacin treatment (group C) Table 4.

DISCUSSION

Higher somatic cell count showed the infection of udder and lead to deterioration of milk quality (Berguland et al., 2007). Normal SCC in buffaloes varies from 0.5×10^5 cells/ml to 3.75×10^5 cells/ml with a mean of 1.4×10^5 cells/ml (Silva and Silva, 1994). At the end of experiment, lowest SCC is seen in the group received the combined therapy of zinc sulphate, copper sulphate and enrofloxacin.

At day 14 post treatment, there was no significant difference in the serum zinc concentration of combined therapy of zinc sulphate and copper sulphate (group D) and zinc sulphate alone therapy (group A).

Due to sub-clinical mastitis in buffaloes, there is decrease in the total leukocyte count as reported by Zaman et al. (1997) and Knight (1983). Similar result of total leukocytes count (4.08×10^6 cells/mm^3) was found in sub-clinically mastitic buffaloes selected in this study at day 0 (before treatment). Normal total

### Table 1: Percentage decrease of Somatic cell count in subclinically mastitic buffaloes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of buffaloes</th>
<th>SCC (×10^5 cells/ml)</th>
<th>Percent (%) decrease in Somatic Cell Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 Mean ± SE</td>
<td>Day 14 PT Mean ± SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A 9</td>
<td>10.06±0.182</td>
<td>4.10±0.099</td>
<td>59.3</td>
</tr>
<tr>
<td>Group B 9</td>
<td>10.12±0.193</td>
<td>5.50±0.181</td>
<td>45.7</td>
</tr>
<tr>
<td>Group C 9</td>
<td>10.06±0.185</td>
<td>4.60±0.140</td>
<td>54.3</td>
</tr>
<tr>
<td>Group D 9</td>
<td>10.13±0.189</td>
<td>2.77±0.169</td>
<td>72.7</td>
</tr>
<tr>
<td>Group E 9</td>
<td>10.26±0.139</td>
<td>10.27±0.177</td>
<td>1% increase in SCC</td>
</tr>
</tbody>
</table>

(PT)= Post Treatment Group A= Zinc sulphate @ 1 g per animal per day for 14 days. Group B= Copper sulphate @ 1 g per animal per day for 14 days. Group C= Enrofloxacin (Encure-10^TM, Nawar Laboratories Pvt. Ltd. Karachi, Pakistan) @ 2.5 mg/kg B.W for 5 consecutive days. Group D= Zinc sulphate @ 1 g per day for 14 days, copper sulphate @ 1 g per day for 14 days and enrofloxacin (Encure-10^TM, Nawar Laboratories Pvt. Ltd. Karachi, Pakistan) @ 2.5 mg/kg B.W for 5 consecutive days. Group E= Untreated control

### Table 2: Bacteriological cure rates percentage of subclinically mastitic buffaloes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number of cured quarters at day 14 post treatment</th>
<th>Cure rate percentage % at day 14 post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A 18</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Group B 13</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Group C 16</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Group D 21</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Group E 2</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Comparison of mean±SE Surf Field Mastitis Test score in sub-clinically mastitic buffaloes

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>3.44±0.18 a</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>3.56±0.18 a</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>3.33±0.17 ab</td>
</tr>
<tr>
<td>D</td>
<td>14</td>
<td>3.44±0.18 a</td>
</tr>
<tr>
<td>E</td>
<td>Mean</td>
<td>3.56±0.18 a</td>
</tr>
</tbody>
</table>

Mean sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 4: Comparison of mean±SE milk yield of affected quarter in sub-clinically mastitic buffaloes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>1.20±0.023 d</td>
</tr>
<tr>
<td>B</td>
<td>14</td>
<td>1.17±0.017 d</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>1.18±0.018 d</td>
</tr>
<tr>
<td>D</td>
<td>14</td>
<td>1.19±0.015 d</td>
</tr>
<tr>
<td>E</td>
<td>14</td>
<td>1.19±0.019 d</td>
</tr>
<tr>
<td>Mean</td>
<td>0</td>
<td>1.19±0.008 B</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

leukocyte count in buffalo is 9.1×10³ cells/mm³ as reported by Hagawane et al. (2009) and Sulong et al. (2008). These treatments help to increase TLC towards normal as at day 14 post treatment. Due to sub-clinical mastitis in buffaloes, there is decrease in the lymphocyte percentage as reported by Zaman et al. (1997) and Knight (1983). Similar result of lymphocyte percentage 47.7% was found in sub-clinically mastitic buffaloes selected in this study at day 0 (before treatment). Normal lymphocyte percentage in buffalo is 56.7% as reported by Hagawane et al. (2009). At day 14 post treatment, lymphocyte percentage is increased towards normal. Surf Field Mastitis Test is a precious tool for semi-quantitative evaluation of milk cell concentration and a good indicator of udder infection (Fazal-ur-Rehman, 1995; Busato et al., 2000). The cure rate of SFMT was nearly similar to the results of Upadhayay et al. (2008) who reported 75% cure rate in zinc sulphate supplementation and 41% cure rate in copper sulphate supplementation. Milk production was almost always lower in sub-clinical mastitis. Buffaloes treated with the combined therapy of zinc sulphate, copper sulphate and enrofloxacin showed maximum recovery in milk yield. These findings are in line with that of Gray and Schalm (1992) who also reported a significant improvement in milk production when cows were recovered from sub-clinical mastitis.

Zinc sulphate supplementation had better results than copper sulphate supplementation. Highest increase in the combined therapy of zinc sulphate, copper sulphate and enrofloxacin (group D) was due to the synergetic effect of zinc sulphate and copper sulphate in the production of lymphocytes and neutrophils (Tomlinson et al., 2008). It can be concluded from the findings of present study that the combined treatment of zinc sulphate, copper sulphate and enrofloxacin was better than zinc sulphate alone, copper sulphate alone and enrofloxacin alone therapy in the treatment of sub-clinically mastitic buffaloes. It may be considered as future line of treatment for mastitis in Pakistan.

REFERENCES


